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## Early impacts of cotton and peanut cropping systems on selected soil chemical, physical, microbiological and biochemical properties

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**Abstract** This study investigated the impacts of cropping systems of cotton (*Gossypium hirsutum* L.; Ct) and peanut (*Arachis hypogaea* L.; Pt) on a Brownfield fine sandy soil (Loamy, mixed, superactive, thermic Arenic Aridic Paleustalfs) in west Texas, United States. Samples (0–12 cm) were taken 2 and 3 years after establishment of the plots from PtPtPt, CtCtPt and PtCtCt in March, June and September 2002, and in March 2003. Soil total N and aggregate stability were generally not different among the cropping systems. The pH of the soils was >8.0. Continuous peanut increased soil organic C, microbial biomass C ( $C_{mic}$ ) and the activities of  $\beta$ -glucosidase,  $\beta$ -glucosaminidase, acid phosphatase, alkaline phosphatase, phosphodiesterase and arylsulfatase compared to the peanut-cotton rotations. The arylsulfatase activity of the fumigated field-moist soil and that resulting from the difference of the fumigated minus non-fumigated soil were greater in PtPtPt, but arylsulfatase activity of non-fumigated soil was unaffected by the cropping systems. Soil  $C_{mic}$  showed a different seasonal variation to enzyme activities during the study. Enzyme activities: microbial biomass ratios indicated that the microbial biomass may not have produced significant amounts of enzymes or that newly released enzymes did not become stabilized in the soil (i.e., due to its low clay and organic matter contents). Fungal (18:2 $\omega$ 6c and 18:1 $\omega$ 9c) and bacterial (15:0, *a*15:0, and *a*17:0) FAMES were higher in PtPtPt than in CtCtPt or PtCtCt cropping systems. Our results suggest that the quality or quantity of residues returned to the soil under a peanut and cotton rotation did not impact the properties of this sandy soil after the first 3 years of this study.

**Keywords** Peanut-cotton rotation · FAME profiles · Enzyme activities · Intracellular arylsulfatase activity · Soil management

### Introduction

Arid lands have been considered agriculturally and economically unimportant, in spite of the fact that the human race depends on their resources (Skujins 1991). With the expected increase in the world population in the twenty-first century, arid lands will have even more significant impacts on the world food supply. For instance, land dedicated to peanut (*Arachis hypogaea* L.) production in the Southern High Plains region of Texas, United States has increased from 17,806 ha to more than 64,980 ha since 1995 (Texas Agricultural Statistics, 1995–2001) providing peanut as a new food crop alternative. Peanut is mainly produced in sandy soils in rotations with cotton (*Gossypium hirsutum* L.), although corn (*Zea mays* L.) and grain sorghum (*Sorghum bicolor* L.) are also excellent crops for rotations with peanut for pathogen prevention (Lemon et al. 2001). Due to the increase in peanut production in west Texas, it is important to investigate and quantify the impacts of cotton and peanut cropping systems on the physical, chemical, microbiological and biochemical properties of sandy soils in order to evaluate long-term sustainability and environmental impacts.

The involvement of microorganisms in organic matter decomposition, nutrient cycling, humus synthesis, soil aggregation and thus, soil function, is well known. The microbial population size and composition control soil processes because they are the main source of enzymes in soils (Tabatabai 1994). Several enzymes are key in organic matter decomposition and in C, N, P, and S nutrient transformation of soil. For example,  $\beta$ -glucosidase activity is key in the last limiting step of cellulose degradation (C cycle) and arylsulfatase activity is important in the organic S mineralization of soil. Little is known about chitin degradation in semiarid soils, but  $\beta$ -glucosaminidase

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activity (Parham and Deng 2000) is a key enzyme involved in the hydrolysis of *N*-acetyl- $\beta$ -D-glucosamine residue from the terminal non-reducing ends of chitooligosaccharides. This hydrolysis is considered to be important to C and N cycling in soils because it participates in the processes whereby chitin is converted to amino sugars, a major source of mineralizable N in soil (Stevenson 1994; Ekenler and Tabatabai 2002). The phosphatases are crucial in organic and inorganic P transformation, but are also significantly affected by soil pH, which controls P availability independent of organic matter content or levels of disturbance.

It has been problematic to interpret enzyme activities because it has not been possible to distinguish the contribution of different enzyme pools to the overall enzyme activity (Nannipieri et al. 2002). Klose and Tabatabai (1999a, b) proposed determination of arylsulfatase activity before and after fumigation of soil with chloroform in order to distinguish the intracellular microbial enzymes from extracellular enzymes stabilized by soil organic matter and clay particles. Limitations of this method are the possible inactivation of the released enzymes by chloroform and its degradation by soil proteases (Klose and Tabatabai 1999a, b; Renella et al. 2002), and the assumption of only extracellular activity in the non-fumigated soil (Renella et al. 2002). Nonetheless, arylsulfatase activity resulting from the difference between the fumigated minus that of non-fumigated soil can give an indication, but not a precise measurement, of the enzyme activity not available to the substrate in the non-fumigated soil, which would be expected to have some relationship with microbial biomass. This information is needed as the microbial biomass and the different pools of enzyme activities may be affected differently by soil management.

Changes observed in soil properties due to crop rotations are related to the quantity and quality of plant residues and nutrients entering the soil, but they will also depend on the soil type. A study with a fine sandy loam soil found no differentiation in the enzyme activities of continuous cotton and a cotton-peanut rotation (Acosta-Martínez et al. 2003a). Studies in loamy soils, with higher clay and organic matter contents than the soils used for peanut production, found that rotations of corn (*Zea mays* L.) with soybean [*Glycine max* (L.) Merr.], oats (*Avena sativa* L.) or meadow (*Medicago sativa* L.) increased microbial biomass, N mineralization, enzyme activities, and organic matter compared to continuous corn (Klose et al. 1999; Klose and Tabatabai 2000; Deng et al. 2000; Moore et al. 2000; Ekenler and Tabatabai 2002). In addition, those studies found that corn in rotations with oats and meadow caused more significant impacts on microbiological properties compared to corn and soybean rotations. In silt and loamy soils, changes in microbial community structure indicated by fatty acid methyl ester (FAME) profiles of the rhizosphere of various plants (Ibekwe and Kennedy 1999) and due to crop rotations (Schutter et al. 2001) have been reported. However, no differences were found in the FAME profiles of sandy

soils under different crop rotations, while there were differences in enzyme activities (Acosta-Martínez et al. 2003a).

The objectives of this study were: (1) to investigate several properties of a sandy soil as affected by cotton and peanut cropping systems including aggregate stability, organic C, total N, soil pH, microbial biomass C, microbial community structure, and enzyme activities; and (2) to assess the seasonal variations of these soil properties.

## Materials and methods

### Sites description and management

This study was conducted on plots established in 2000 on a Brownfield fine sandy soil (loamy, mixed, superactive, thermic Arenic Aridic Paleustalfs) with different cotton (*Gossypium hirsutum* L.; Ct) and peanut (*Arachis hypogaea* L.; Pt) cropping systems and irrigation level treatments at the Western Peanut Growers Research Farm (WPGRF) in Gaines County, Texas. The plots were established to provide a better understanding of peanut water requirements, and to test irrigation methods for growing peanuts in the typical soil-climate-topography conditions in west Texas. The soil contains 7% clay, 2% silt, and 91% sand. The cropping systems we investigated were summer crops in 2000, 2001 and 2002, without a winter cover crop, such as continuous peanut (PtPtPt), cotton-cotton-peanut (CtCtPt), and peanut-cotton-cotton (PtCtCt).

The field study was deep tilled (60 cm) and bedded in circular rows in the springs of 2000 and 2001. In 2002, the land area that had been under peanuts the previous year and was to be planted with cotton was deep tilled to reduce problems with volunteer peanuts. The plots under continuous peanut, continuous cotton, and peanut following cotton were plowed to a depth of 20 cm using a field cultivator and re-bedded. Beds were spaced at 90 cm. The cotton cultivar used was Paymaster 2326RR, and the peanut variety was Flavor Runner 458. Nitragin Implant granular inoculant was applied using granular insecticide application equipment on the planter. In all years, ethalfluralin (Sonalan; 1.75 l ha<sup>-1</sup>) was used as the peanut preplant herbicide. Post-emergence herbicides (including Benzothiazol/Diphenylether, Storm; Bentazon, Basagran; and others) were used on peanuts as needed. In 2000, Pendimethalin (Prowl; 1.75 l ha<sup>-1</sup>) was used as the preplant cotton herbicide. In 2001 and 2002, Trifluralin (Trifluralin; 1.46 l ha<sup>-1</sup>) was used as the preplant cotton herbicide. Post-emergence applications of glyphosate (Roundup; 1.9 l ha<sup>-1</sup>) were made to cotton.

Irrigation was conducted twice weekly by doing alternate furrows with rates targeted to achieve the desired percent replacement of evapotranspiration (ET) demand with the low energy precision application (LEPA) method. In 2000, LEPA was the predominant irrigation application method, with other applicator types, e.g., low elevation spray applicators (LESA) or wobblers, used in selected experimental areas. In 2001 and 2002, LESA became the dominant application method, with LEPA and wobblers on selected areas. In season, irrigation applications were approximately 36, 53, and 71 cm for 50%, 75% and 100% ET treatments, respectively, with variable application rates applied from July to September each season. The irrigation rate treatments began in the growing season of 2001 for continuous peanut and in 2002 for the cotton and peanut rotations. Fertilizer was applied to cotton and peanut according to the crop requirements and soil tests by ground application and/or through the center pivot irrigation system.

## Soil sampling

Preliminary studies in our laboratories showed that irrigation rates did not affect the microbial populations and activities in this sandy soil at the point of the study. However, three soil samples (0–12 cm) were taken from each of the three ET replacement irrigation levels (50%, 75%, and 100%) used for CtCtPt or PtCtCt each sampling. For PtPtPt, three samples were collected randomly from the different irrigation levels each sampling, and a statistical test showed no effects of irrigation rates at the end of the study. Each soil sample was a composite mixture of four locations of a row using a 5-cm diameter probe core sampler. Each sampling, the two field replicates available for the cropping systems were sampled. Samplings occurred in March, June, and September 2002, and in March 2003. The samples were kept at 4°C until soil microbiological analysis was performed, the same month of the sampling, and a subset was air-dried for other analyses. Soil moisture was determined after drying at 105°C for 48 h.

## Soil chemical and physical analyses

Soil pH was measured on the air-dried soil using a glass combination electrode (soil:water ratio, 1:2.5). Soil organic C and total N contents were determined on the air-dried soil using the Vario Max-ELEMENTAR CN-analyzer (D-63452 Hanau, Germany). Soil aggregate stability was determined on 2 g soil (<1–2 mm air-dried aggregates) by the method described by Kemper and Rosenau (1986).

## Soil microbiological and biochemical analyses

### Enzyme activities

The activities of  $\beta$ -glucosidase,  $\beta$ -glucosaminidase, alkaline and acid phosphatase, phosphodiesterase, and arylsulfatase were assayed using 1 g air-dried soil (not sieved) without toluene, with their appropriate substrate and incubated for 1 h at their optimal pH. The assay procedures have been described elsewhere:  $\beta$ -glucosaminidase activity in Parham and Deng (2000), and the other enzyme activities in Tabatabai (1994). The enzyme activities were assayed in duplicate with one control, and the results were expressed in mg of *p*-nitrophenol (PN) released kg<sup>-1</sup> soil (moisture-free basis) h<sup>-1</sup>.

### Microbial biomass C

The microbial biomass C ( $C_{mic}$ ) was determined on a 15-g oven-dry equivalent field-moist soil sample (not sieved) by the chloroform-fumigation-extraction method (CFEM) described by Vance et al.

(1987), using 0.5 M K<sub>2</sub> SO<sub>4</sub> as an extractant. The organic C was quantified on the fumigated and non-fumigated (control) soil by a C analyzer (Schimadzu Model TOC-V/CPH-TN). Biomass C was calculated using a  $k_{EC}$  factor of 0.45 (Wu et al. 1990).

### Arylsulfatase activity of the fumigated and non-fumigated soil

According to Klose and Tabatabai (1999a), arylsulfatase activity was determined (Tabatabai 1994) in a set of field-moist soil samples fumigated with chloroform for 24 h, and in non-fumigated field-moist soil, both in the absence of toluene. The difference between the arylsulfatase activity of the chloroform-fumigated soil and the non-fumigated soil was then calculated.

## FAME profiles

Fatty acids were extracted from the soils using the procedure described for pure culture isolates by the Microbial Identification System (MIS, Microbial ID, Newark, Del.) as previously applied for soil analyses (Cavigelli et al. 1995; Ibekwe and Kennedy 1999; Acosta-Martínez et al. 2003a). Briefly, the method consists of four steps: (1) saponification of fatty acids in soil (3 g not sieved field-moist) with 3 ml 3.75 M NaOH (methanol:water, 1:1) solution under heat (100°C) for 30 min; (2) methylation of fatty acids by adding 2 ml 6 M HCl in aqueous methanol (1:0.85) under heat (80°C) for 10 min; (3) extraction of the FAMES with 3 ml 1:1 hexane: methyl-*tert*-butyl-ether solution and rotating the samples end-over-end for 10 min; and (4) washing of the organic phases with 1.2% diluted NaOH by rotating the tubes end-over-end for 5 min. The FAMES were analyzed in a 6890 GC Series II (Hewlett Packard, Wilmington, Del.) equipped with a flame ionization detector and a 25-m  $\times$  0.2-mm fused silica capillary column using ultra high purity hydrogen as the carrier gas. The temperature program was ramped from 170 to 250°C at 5°C min<sup>-1</sup>. The FAMES were identified, and their relative peak areas determined by the MIS Aerobe method of the MIDI system (Microbial ID, Newark, Del.). Each sample peak was compared to standard fatty acids and interpolation of retention time was done using the equivalent chain length method. The FAMES are described by the number of C atoms, followed by a colon, the number of double bonds and then by the position of the first double bond from the methyl ( $\omega$ ) end of molecules, and *cis* and *trans* isomers are indicated by *c* or *t*, respectively. Branched fatty acids are indicated by the prefixes *i* and *a* for iso and anteiso, respectively.

## Statistical analyses

Analysis of variance (ANOVA), multivariate analysis of variance (MANOVA), and mean separation by least significant differences

**Table 1** Selected chemical and physical properties in soils under different cotton and peanut cropping systems

System <sup>a</sup>	pH <sup>b</sup>				Organic C (g kg <sup>-1</sup> )				Total N (g kg <sup>-1</sup> )				Aggregate stability <sup>c</sup> (%)			
	2002			2003	2002			2003	2002			2003	2002			2003
	March	June	Sept.	March	March	June	Sept.	March	March	June	Sept.	March	March	June	Sept.	March
PtPtPt	8.3b <sup>d</sup>	8.2b	8.0b	8.1a	1.69a	1.71a	1.79a	2.09a	0.20a	0.20a	0.22a	0.28a	4.86a	3.05a	3.00a	3.01a
PtCtCt	8.6a	8.7a	8.5a	8.0a	1.39b	1.53b	1.67ab	1.44b	0.18ab	0.17a	0.22a	0.29a	4.18a	3.10a	4.34a	4.33a
CtCtPt	8.4ab	8.1b	8.5a	8.2a	1.41b	1.57ab	1.39b	1.45b	0.17b	0.21a	0.23a	0.22b	5.11a	4.34a	4.54a	4.00a

<sup>a</sup> Pt Peanut, Ct cotton. Two year rotation for March and June 2002. Three year rotation for September 2002 and March 2003

<sup>b</sup> The pH was determined in a soil:water ratio of 1:2.5

<sup>c</sup> According to the method of Kemper and Rosenau (1986): [stable soil aggregates/(unstable soil aggregates + stable soil aggregates)]  $\times$  100

<sup>d</sup> Values with similar letter means the differences of the effects of cropping systems are not significant at the 0.05 level

(LSD) at  $P < 0.05$  significant levels among the treatments were performed using the general linear model (GLM) procedure of the SAS system (1999). Principal component analyses (PCA) were performed in the SAS system to demonstrate the similarities and differences in FAME profiles among samples due to sampling time by including all the fatty acids extracted.

## Results and discussion

### Physical and chemical properties

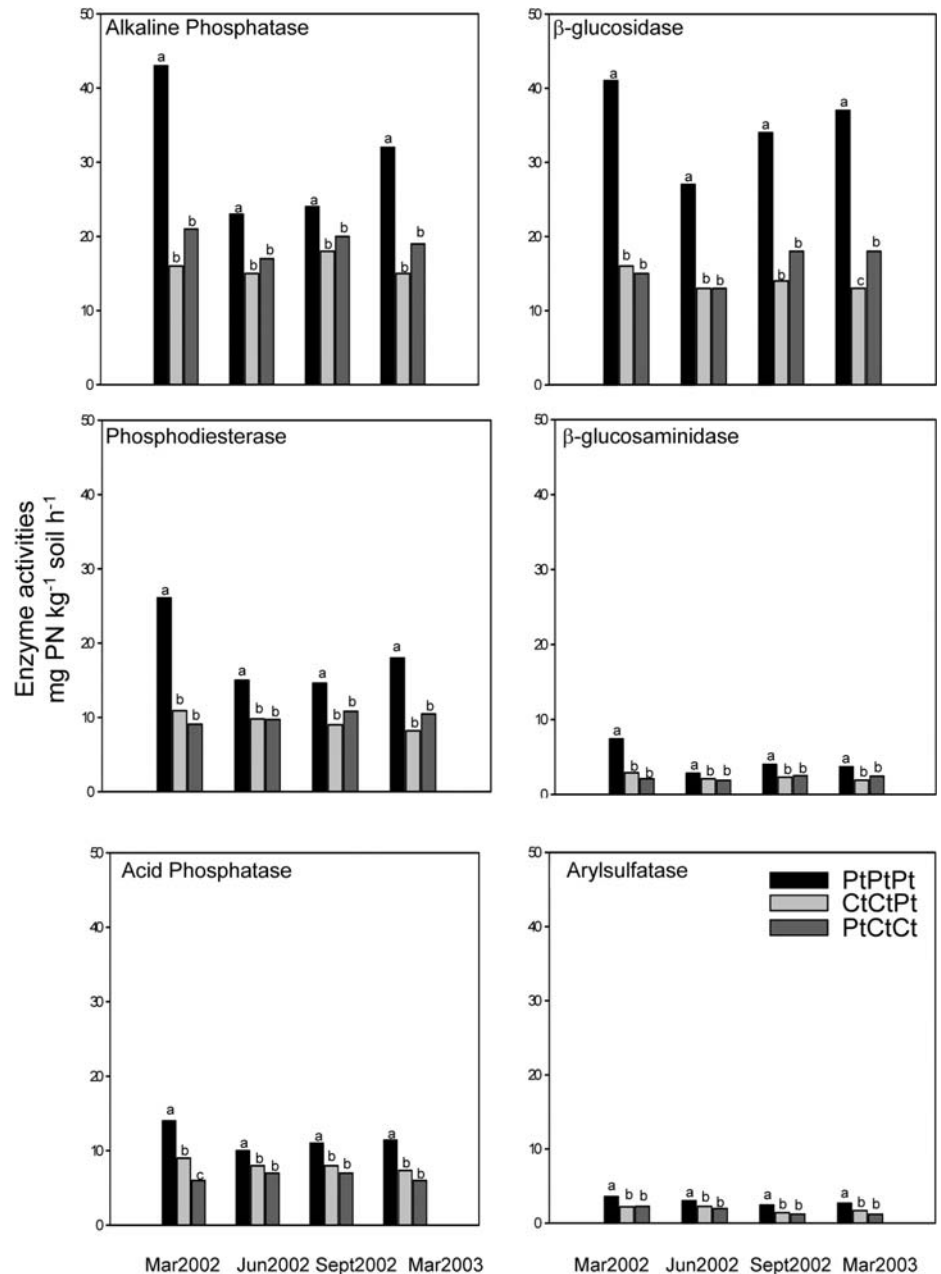
Lower soil pH was found in PtPtPt compared to either PtCtCt or CtCtPt in September 2002 (Table 1). In March 2003, a decrease in soil pH was found in PtCtCt and CtCtPt. The soil pH ranged from 8.0 to 8.3 in PtPtPt, and

from 8.0 to 8.7 in PtCtCt and CtCtPt. Because the pH was generally higher than 8.0 (alkaline) in this sandy soil, it is not expected that the lower soil pH of PtPtPt in September will significantly impact the microbiological and biochemical properties compared with PtCtCt and CtCtPt.

Organic C was higher in soils under PtPtPt compared with that under PtCtCt or CtCtPt in March 2002 and 2003. There were no differences between the PtCtCt and CtCtPt (Table 1). Perhaps the low organic matter content typical of sandy soils caused the already significant increase observed in the soil organic C content under PtPtPt. Soil total N was not generally different among cropping systems, except for March 2002 and 2003.

Among physical assessments, it was considered most practical to only investigate the aggregate stability of this

**Fig. 1** Enzyme activities under different peanut and cotton cropping systems in a semiarid sandy soil over a 1-year period. The activities were determined on air-dried soil





sandy soil. Changes in aggregate stability are believed to provide a long-term indication of changes in organic matter quantity and quality (Lynch and Bragg 1985). The aggregate stability of this soil was low, as typical for sandy soils, it showed no differences among cropping systems, and it did not differ significantly in this 1-year study.

#### Microbial and biochemical properties: enzyme activities

Previous studies in the same semiarid region found similar  $\beta$ -glucosaminidase,  $\beta$ -glucosidase, arylsulfatase, and alkaline phosphatase activities in peanut-cotton rotations compared to continuous cotton in a fine sandy loam (Acosta-Martínez et al. 2003a), but information was not available on the comparison of peanut-cotton rotations and continuous peanut. During this 1-year study, following the trend of the organic C content, enzyme activities were higher in PtPtPt compared with those under PtCtCt or CtCtPt, and there were usually no differences between PtCtCt and CtCtPt in this sandy soil (Fig. 1). The enzyme activities investigated were inter-correlated in all sampling times (Table 2). Our early assessment appears to indicate that continuous peanut (legume) was the only cropping system that impacted the enzyme activities involved in C, N, P and S mineralization investigated in this soil. Among the enzyme activities, the higher  $\beta$ -glucosaminidase activity in PtPtPt provides an indication of changes in the N cycling of this sandy soil because this enzyme activity has been positively correlated to N mineralization in soils (Ekenler and Tabatabai 2002). The higher enzyme activities of PtPtPt, especially  $\beta$ -glucosaminidase activity, must have been influenced by the continuous  $N_2$  fixation under this monoculture system. In addition, mycorrhizal associations are key in nutrient cycling (Barea 1991; Allen 1992), and are known to increase secretion of phosphatases.

Similar enzyme activities in PtCtCt and CtCtPt in all samplings indicated there was no influence on microbial

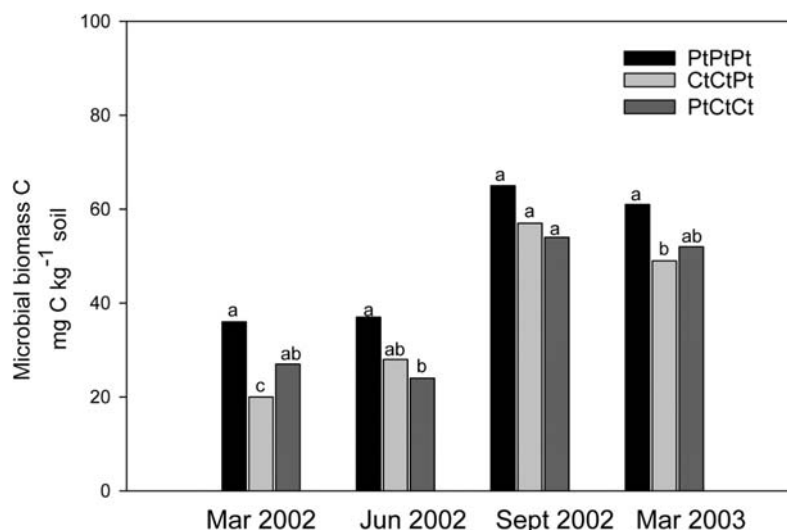
populations and activities due to the crop sequence or crop type at any sampling for this sandy soil. In contrast to our findings, another study found that the microbial biomass C and the same enzymes activities we studied were affected by which crop was sampled in the rotation (Acosta-Martínez et al. 2004). Previous work with several enzyme activities found that  $\beta$ -glucosidase and dehydrogenase activities showed differences between soils under corn and soybean (i.e., higher in corn), whereas phosphatase and arylsulfatase activities did not differ between crops (Bergstrom and Monreal 1998).

In monitoring seasonal changes during this 1-year study, there was a decrease in soil enzyme activities from March to June that was only observed in PtPtPt, and thus, perhaps indicating differences in seasonal variations among the cropping systems. In addition, comparison among the enzymes studied showed that acid phosphatase activity was always three times lower than alkaline phosphatase activity in the cropping systems, which is due to the alkaline pH of this sandy soil. These phosphatases are inductive enzymes and their synthesis is strongly affected by soil pH (Eivazi and Tabatabai 1977; Acosta-Martínez and Tabatabai 2000). The values of the enzyme activities found are within the lower ranges reported for different soils, except for alkaline phosphatase activity which was higher than the ranges reported for other soils probably attributable to the high soil pH (>8.0) of this typical semiarid soil (Nannipieri et al. 2002; Acosta-Martínez et al. 2003b).

#### Microbial and biochemical properties: microbial biomass C

Similar to the enzyme activities,  $C_{mic}$  was higher in soils under PtPtPt compared to either PtCtPt or PtCtCt in March and June 2002 (Fig. 2). In September, however, there were no significant differences among cropping systems, but there were significant differences between PtPtPt and CtCtPt in March 2003. These findings are not in

**Fig. 2** Microbial biomass C under different peanut and cotton cropping systems in a semi-arid sandy soil over a 1-year period



**Table 2** Correlations ( $r$ )<sup>a</sup> between the soil chemical, microbiological, and biochemical parameters investigated

Soil parameters <sup>b</sup>	Sampling times			
	March 2002	June 2002	Sept. 2002	March 2003
Organic C and:				
Microbial biomass C	N.S.	0.64***	0.76***	0.55***
$\beta$ -Glucosidase activity	0.64***	0.86***	0.88***	0.87***
$\beta$ -Glucosaminidase activity	N.S.	0.92***	0.78***	0.54***
Phosphodiesterase activity	0.56***	0.78***	0.91***	0.81***
Alkaline phosphatase activity	0.62***	0.41**	0.87***	0.81***
Acid phosphatase activity	0.48***	0.42**	0.82***	0.68***
Aryls. act-(f), -(nf), -(f-nf)	0.75***, -0.59***, 0.43**	0.60***, N.S., 0.52***	0.78***, N.S., 0.56***	0.90***, N.S., 0.62***
Total N and:				
Microbial biomass C	N.S.	0.72***	N.S.	0.55***
$\beta$ -Glucosidase activity	0.65***	0.89***	N.S.	0.65***
$\beta$ -Glucosaminidase activity	N.S.	0.79***	N.S.	0.35*
Phosphodiesterase activity	0.56***	0.81***	N.S.	0.52***
Alkaline phosphatase activity	0.64***	0.64***	N.S.	0.60***
Acid phosphatase activity	0.35*	N.S.	N.S.	N.S.
Aryls. act-(f), -(nf), -(f-nf)	0.68***, -0.63***, 0.46***	0.80***, N.S., 0.75***	0.30*, N.S., 0.30*	0.55***, N.S., 0.88***
Microbial biomass C and:				
$\beta$ -Glucosidase activity	0.33*	0.35*	N.S.	0.48**
$\beta$ -Glucosaminidase activity	N.S.	0.41**	0.40**	0.37*
Phosphodiesterase activity	0.49***	N.S.	N.S.	0.54***
Alkaline phosphatase activity	0.34*	N.S.	0.31*	0.47**
Acid phosphatase activity	N.S.	N.S.	N.S.	N.S.
Aryls. act-(f), -(nf), -(f-nf)	0.60***, N.S., 0.60***	N.S., N.S., N.S.	N.S., N.S., N.S.	0.47***, N.S., 0.56***
$\beta$ -Glucosidase activity and:				
$\beta$ -Glucosaminidase activity	0.84***	0.69***	0.61***	0.49***
Phosphodiesterase activity	0.91***	0.70***	0.45**	0.83***
Alkaline phosphatase activity	0.72***	0.70***	0.59***	0.88***
Acid phosphatase activity	0.55***	0.38*	0.58***	0.39*
Aryls. act-(f), -(nf), -(f-nf)	0.40**, N.S., 0.44**	0.69***, N.S., 0.67***	0.69***, N.S., 0.66***	0.74***, N.S., 0.79***
$\beta$ -Glucosaminidase activity and:				
Phosphodiesterase activity	0.90***	0.51***	0.52***	0.49***
Alkaline phosphatase activity	0.74***	0.36*	0.67***	0.54***
Acid phosphatase activity	0.53***	0.46**	0.49***	N.S.
Aryls. act-(f), -(nf), -(f-nf)	0.60***, N.S., 0.62***	0.47***, 0.33*, 0.38*	0.68***, 0.43***, 0.60***	0.42***, N.S., 0.41***
Phosphodiesterase activity and:				
Alkaline phosphatase activity	0.78***	0.74***	0.44***	0.85***
Acid phosphatase activity	0.53***	N.S.	0.54***	0.54***
Aryls. act-(f), -(nf), -(f-nf)	0.63***, N.S., 0.66***	0.74***, 0.33*, 0.67***	0.70***, 0.42***, 0.62***	0.75***, N.S., 0.81***
Alkaline phosphatase activity and:				
Acid phosphatase activity	0.47***	N.S.	0.40***	0.46***

**Table 2** (continued)

Soil parameters <sup>b</sup>	Sampling times			
	March 2002	June 2002	Sept. 2002	March 2003
Aryls. act-(f), -(nf), -(f-nf)	0.53***, N.S., 0.61***	0.70***, N.S., 0.71***	0.64***, 0.52***, 0.54***	0.77***, N.S., 0.82***
Acid phosphatase activity and:				
Aryls. act-(f), -(nf), -(f-nf)	0.37*, N.S., 0.37*	N.S., N.S., N.S.	0.37*, N.S., 0.32*	0.56***, N.S., 0.49***

<sup>a</sup> \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; N.S. not significant.

<sup>b</sup> Correlations between the first property and the properties following ( $n = 42$ ). *Aryls. act* Arylsulfatase activity, (f) fumigated soil, (nf) non-fumigated soil

agreement with previous increases observed in microbial biomass C and N under crop rotations in comparison to monoculture systems (Moore et al. 2000). The increase of microbial biomass and enzyme activities generally observed under crop rotations are due to the variations in the amount of crop residues left in the soil, on the decomposition rates of the residue material, and, thus, in their contribution to an easily degradable soil organic matter pool in comparison to monoculture systems (Janzen and Lucey 1988; Wienhold and Halvorson 1998; Klose et al. 1999; Klose and Tabatabai 2000; Ekenler and Tabatabai 2002). In addition, long-term field experiments with other soil types showed that including legumes in crop rotations increased the organic C and N, and thereby the soil microbial biomass and enzyme activities (Bolton et al. 1985). Thus, the higher  $C_{mic}$  and enzyme activities found in PtPtPt in comparison to CtCtPt and PtCtCt in this study are explained by the fact that the monoculture system was based on a legume crop and that cotton incorporates lower amounts of crop residues in the rotation than peanut.

From June to September 2002, soil  $C_{mic}$  was generally increased. The enzyme activities, however, did not reflect the increase found for soil  $C_{mic}$  in September 2002 and maintained in March 2003 (Table 2). These results may indicate that the rhizosphere effects (i.e., increased plant growth and root secretions) influenced the soil microbial populations but not their production of enzymes. In fact, the enzyme activity: $C_{mic}$  ratios showed a decrease from June to September in all cropping systems demonstrating that any increase in the microbial biomass did not produce higher enzyme activities per unit of biomass (Table 3). For this sandy soil, this finding may be attributed to degradation or inactivation of the newly released arylsulfatases and/or extracellular arylsulfatases before they were stabilized in soil (i.e., due to its low clay and organic matter contents). Multivariate statistical analyses showed that the enzyme activity: $C_{mic}$  ratios were affected by sampling time, but they were not statistically different among cropping systems.

The  $C_{mic}$  values found for this sandy soil were lower than those reported for other soils and vegetation types of humid regions (Klose et al. 1999; Moore et al. 2000) or a semiarid soil with higher clay content (Acosta-Martínez et al. 2004), which demonstrates the key role of the clay-

organic matter complexes in supporting microbial populations and stabilizing enzymes in soil.

Microbial and biochemical properties: arylsulfatase activity of chloroform-fumigated and non-fumigated soil

The microbial biomass is the main source of intracellular and extracellular enzymes in soils (Tabatabai 1994), but traditional soil enzyme assays do not distinguish between these main enzyme pools (Nannipieri et al. 2002). This may explain why, while enzyme activities and microbial biomass C were significantly and positively correlated in March 2002 and 2003, these properties were not correlated in June and September 2002 (Table 2). Contradictory results have been found in the relationship between enzyme activities and microbial biomass in soils because enzyme activities are constitutive or inducible, and in the latter case, their synthesis can be induced by specific compounds (Klose et al. 1999; Dilly and Nannipieri 2001). Recent work has shown that the contribution of the different pools of enzyme activities to the overall enzyme activity in soils depends on the enzyme and soil properties (Klose et al. 1999; Klose and Tabatabai 1999a,b; Acosta-Martínez et al. 2004). In an attempt to explain the low correlation of soil enzyme activities and microbial biomass in June and September, we used the chloroform-fumigation method of Klose and Tabatabai (1999a) to have an indication of intracellular arylsulfatase activity even if this method, as discussed above, provides only an estimate of the intra-cytoplasmic enzyme activity pool. Indeed, Renella et al. (2002) suggested a complete inhibition of active proteases to determine the intracellular activity with the method of Klose and Tabatabai (1999a). In addition, it has been recognized that the enzyme activity of the non-fumigated soil may not represent only the extracellular enzymes (Klose and Tabatabai 1999a; Renella et al. 2002). In our study, arylsulfatase activity of the fumigated field-moist soil (assumed total activity), and the activity of the fumigated soil minus non-fumigated soil (assumed intracellular activity) were higher in PtPtPt compared to CtCtPt and PtCtCt (Fig. 3). These results are correlated with the microbial biomass and other enzyme activities (Table 2).

**Table 3** Enzyme activities: microbial biomass C ratio of the soil over time

Ratio <sup>a</sup>	Enz. act.:C <sub>mic</sub> <sup>b</sup>	Time <sup>c</sup>
$\beta$ -glucosidase act.:C <sub>mic</sub>		***
Mar. 2002	1.36	
June 2002	0.85	
Sept. 2002	0.50	
Mar. 2003	0.45	
$\beta$ -glucosaminidase act.:C <sub>mic</sub>		***
Mar. 2002	0.23	
June 2002	0.12	
Sept. 2002	0.06	
Mar. 2003	0.05	
Phosphodiesterase act.:C <sub>mic</sub>		***
Mar. 2002	0.23	
June 2002	0.12	
Sept. 2002	0.06	
Mar. 2003	0.05	
Alkaline phosphatase act.:C <sub>mic</sub>		***
Mar. 2002	1.52	
June 2002	0.99	
Sept. 2002	0.48	
Mar. 2003	0.45	
Acid phosphatase act.:C <sub>mic</sub>		***
Mar. 2002	0.63	
June 2002	0.47	
Sept. 2002	0.18	
Mar. 2003	0.16	
Aryls. act (f): C <sub>mic</sub>		***
Mar. 2002	0.31	
June 2002	0.43	
Sept. 2002	0.16	
Mar. 2003	0.14	
Aryls. act (nf):C <sub>mic</sub>		***
Mar. 2002	0.14	
June 2002	0.15	
Sept. 2002	0.06	
Mar. 2003	0.07	
Aryls. act (f-nf):C <sub>mic</sub>		***
Mar. 2002	0.15	
June 2002	0.28	
Sept. 2002	0.10	
Mar. 2003	0.07	

<sup>a</sup> C<sub>mic</sub> Microbial biomass, Aryls. act arylsulfatase activity, (f) fumigated soil, (nf) non-fumigated soil

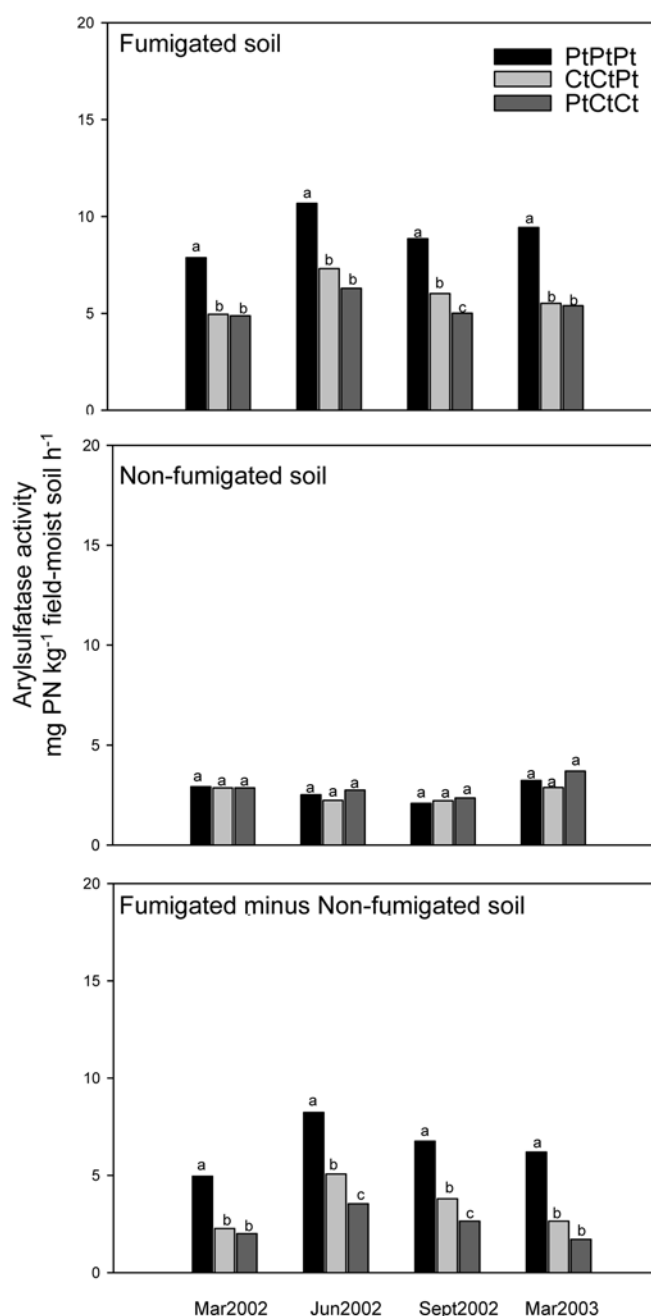
<sup>b</sup> The ratio of soil enzyme activities and C<sub>mic</sub> was calculated for the three cropping systems because the effect of cropping system was not significant

<sup>c</sup> Multivariate analyses of variance for the effect of time. \*\*\*  $P < 0.001$

In contrast, arylsulfatase activity of the non-fumigated field-moist soil was unaffected by the cropping systems throughout the whole study and it was not correlated to soil organic C (Table 2). In our study, assuming arylsul-

fatase activity of the non-fumigated field-moist soil was extracellular, would not be in agreement with the arylsulfatase activity of the air-dried soil, which was affected by the cropping systems (Fig. 1). Thus, the results suggest that the chloroform-fumigation of field-moist soil as well as air-drying soil released enzymes located in the microbial cell cytoplasm that contributed to the higher total arylsulfatase activity of PtPtPt.

Klose et al. (1999) and Klose and Tabatabai (1999a) reported that the assumed intracellular arylsulfatase activity (activity of microbial biomass) averaged 57.7% of the



**Fig. 3** Arylsulfatase activity of the fumigated soil, non-fumigated soil, and fumigated minus non-fumigated soil under different peanut and cotton cropping systems in a semiarid sandy soil over a 1-year period. The activities were determined on field-moist soil



overall enzyme activity in ten Iowan soils that differed in organic C, and sand and clay contents. In our study, the assumed intracellular activity (difference of the fumigated and non-fumigated soil) represented 33–57% of the overall

arylsulfatase activity. However, four of the ten soils studied by Klose and Tabatabai (1999a), and studies with other soils, have found higher contributions of the

**Table 4** Fatty acid methyl ester (FAME) composition in soils as affected by the cropping systems and time

Fatty acid <sup>a</sup>	Cropping systems			MANOVA <sup>b</sup>	
	PtPtPt (%)	PtCtCt (%)	CtCtPt (%)	Time	Cropping system
Fungi indicators					
18:1 $\omega$ 9c				N.S.	***
Mar. 2002	13.45	9.37	11.49		
June 2002	12.04	11.11	11.33		
Sept. 2002	13.34	10.31	10.01		
Mar. 2003	15.78	12.06	9.31		
18:2 $\omega$ 6c				N.S.	***
Mar. 2002	12.29	9.00	9.03		
June 2002	9.71	9.94	8.83		
Sept. 2002	10.97	8.79	7.80		
Mar. 2003	11.04	10.00	6.72		
18:3 $\omega$ 6c				***	N.S.
Mar. 2002	1.48	1.95	1.74		
June 2002	0.37	0.35	0.14		
Sept. 2002	0.59	0.25	0.07		
Mar. 2003	0.63	0.23	0.28		
Bacteria indicators					
15:0				***	**
Mar. 2002	0.49	0.20	0.10		
June 2002	0.73	0.56	0.44		
Sept. 2002	0.48	0.47	0.59		
Mar. 2003	0.54	0.60	0.39		
i15:0				***	N.S.
Mar. 2002	3.90	4.27	4.07		
June 2002	4.21	4.33	4.67		
Sept. 2002	4.93	4.85	4.86		
Mar. 2003	3.63	3.62	3.63		
a15:0				**	***
Mar. 2002	3.78	2.89	2.66		
June 2002	2.96	2.61	2.77		
Sept. 2002	3.91	2.76	2.51		
Mar. 2003	2.67	2.50	2.21		
i17:0				**	N.S.
Mar. 2002	0.99	1.05	1.32		
June 2002	1.24	1.33	1.44		
Sept. 2002	1.53	1.39	1.59		
Mar. 2003	1.18	1.31	0.98		
i16:0				***	N.S.
Mar. 2002	1.67	1.53	1.96		
June 2002	1.79	2.11	2.26		
Sept. 2002	2.18	2.19	2.25		
Mar. 2003	1.87	2.04	1.49		
a17:0				***	*
Mar. 2002	0.82	0.39	0.34		
June 2002	0.99	0.97	0.83		
Sept. 2002	1.03	0.94	1.11		
Mar. 2003	0.99	1.07	0.73		

<sup>a</sup> Pt Peanut, Ct cotton. Two year rotation for March and June 2002. Three year rotation for September 2002 and March 2003

<sup>b</sup> Multivariate analyses of variance for the effect of time or cropping system: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

assumed intracellular activity with respect to the total activity (Acosta-Martínez et al. 2004; Li and Sarah 2003).

The assumed total and intracellular arylsulfatase activities were in the lower ranges of values reported for other soils (Klose and Tabatabai 1999a; Klose et al. 1999; Acosta-Martínez et al. 2004). These results were expected because of the low surface area of sandy soils, which will tend to sustain lower microbial biomass and enzyme activities, especially because they have a lower capacity to stabilize enzymes than soils with higher clay and organic matter contents. Under the low organic matter, clay, and soil moisture contents of semiarid soils, enzymes may be more easily denatured by high temperatures and salt contents (Li and Sarah 2003).

#### Microbial and biochemical properties: FAME profiles

The FAME analysis of soil has been suggested for the characterization of microbial community structure as a microbiological indicator of soil quality changes (Turco et al. 1994; Kennedy 1999; Schutter et al. 2001). FAME analysis presents an advantage to culturing methods because it avoids selectivity toward fast growing microorganisms in the media, but it may not provide a current assessment of the microbial community structure because it can extract fatty acids from clay-organic matter complexes. However, fatty acid extraction from clay-organic matter complexes may be more significant for soils with high clay and organic matter contents than for sandy soils.

Bacteria and fungi abundance in the rhizosphere are influenced by nutrient status of both plant and soil (Kennedy 1999). The MANOVA conducted for nine fatty acids suggested as either fungi or bacteria indicators (Vestal and White 1989) showed significant differences in their relative abundance among the cropping systems of cotton and peanut evaluated (Table 4). The relative abundance of the fungi fatty acids 18:2 $\omega$ 6c and 18:1 $\omega$ 9c were generally higher in PtPtPt compared to CtCtPt and PtCtCt during the study. The bacteria fatty acids 15:0, *a*15:0, and *a*17:0 were generally higher in PtPtPt than in CtCtPt and PtCtCt in March and June 2002. The different soil microbial community structure under PtPtPt may reflect the impacts of the continuous root secretion of nutrients by the typical peanut (legume) root symbiotic association with rhizobia and VAM fungi compared with the interrupted situation when peanut was in rotation with cotton. The higher contents of fungi and bacteria indicator fatty acids in continuous peanut are in agreement with the higher microbial biomass and enzyme activities that were also found.

PCA accounted only for 37% of the total variability among samples, but suggested separation of the FAME profiles of June 2002 in comparison to the other sampling times (not shown). MANOVA showed significant differences in the relative abundance of the nine fatty acid indicators of fungi and bacteria evaluated due to time of sampling, except for 18:1 $\omega$ 9c and 18:2 $\omega$ 6c, which

remained unchanged over time (Table 4). However, there were no definitive trends for these individual fatty acids due to time of sampling. The separation of FAME profiles in June from the other samplings could be explained by the higher temperatures and low precipitation that generally occurs in summer in comparison to spring and fall in semiarid regions.

Our findings for this sandy soil are in agreement with other studies that have found changes in FAME profiles due to seasonal variations (Schutter et al. 2001), and management (Klug and Tiedje 1993; Cavigelli et al. 1995; Ibekwe and Kennedy 1999; Schutter et al. 2001), except that in our early assessment continuous peanut showed higher fungi and bacteria compared to cotton and peanut rotations. Crop rotations have been recognized as a key component in sustainable systems because they are beneficial to microbes, interrupt the cycle of pathogens, reduce weed populations and thus promote more diversified microbial community structure in the long-term when compared to continuous monoculture (Kennedy 1999). The differences observed in soil rhizosphere populations under crop rotations compared to continuous monoculture systems are due to variations in the concentrations and types of organic compounds released by roots of different plants (Lynch and Bragg 1985; Kennedy 1999). Legumes are beneficial in a rotation because they supply symbiotically fixed N to the system, aid in maintaining proper water status, and reduce pathogen load (Kennedy 1999). Previous work has reported that monocropping peanut tends to reduce yields and increases production costs and diseases such as pod rots, southern blights, and sclerotinia blight (Lemon et al. 2001). Thus, the sustainability problems of continuous monoculture systems suggest that within the increases of fungal and bacterial FAMEs in soils under PtPtPt, increases in pathogenic microorganisms may have occurred, without it being possible to detect this with FAME analyses.

#### Summary and conclusions

Organic C, microbial biomass, enzyme activities, and fungi and bacteria populations of soil were higher under PtPtPt compared to PtCtCt or CtCtPt cropping systems in our early assessment. However, it is known that monoculture continuous practices are not a long-term sustainable system, and thus, this study indicated that changes or increases in the soil properties investigated, although important in soil function, are not always an indication of plant productivity and/or sustainability of the soil-cropping system. Our results suggest that the quality or quantity of residues returned to the soil under a peanut and cotton rotation did not impact the properties of this sandy soil after the first three years of this study. The results are most likely due to the combination of low residue incorporation with a cotton crop in comparison to peanut and to the high sand and low clay-organic matter contents of this soil, and thus, low soil surface area of the soil. Because continuous peanut is not a long-term sustainable

system, our findings may provide indications that other crops, perhaps sorghum or corn, may be more suitable to be used in rotations with peanut in order to impact the function and quality of sandy soils.

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